

WHAT IS CLAIMED IS:

1. A native, authentic, enzymatically active NTPase/RNA helicase protein produced by a process comprising the steps of:

- 5 a) expressing an NTPase/RNA helicase
 encoding nucleic acid of hepatitis C
 virus in a eukaryotic expression system
 such that a complete, authentic and
10 native NTPase/RNA helicase protein is
 synthesized, said authentic and native
 NTPase/RNA helicase protein comprising
 amino acids 1027 -1657;
 - 15 b) extracting NTPase/RNA helicase protein
 from said eukaryotic expression system
 in an enzymatically active form of said
 protein; and
 - 20 c) purifying said NTPase/RNA helicase
 protein such that the enzymatically
 active form of said protein is
 maintained.
- 25 2. The protein produced according to claim 1,
 said nucleic acid of hepatitis C virus in step a)
 corresponding to a human hepatitis C virus nucleic
 acid.
- 30 3. The protein produced according to claim 1,
 said nucleic acid of hepatitis C virus in step a) being
 derived from a genotype of the human hepatitis C virus
 nucleic acid.
- 35 4. The protein produced according to claim 1,
 wherein said nucleic acid of hepatitis C virus in step

a) is a variant of the human hepatitis C virus.

5 5. The protein produced according to claim 1,
said nucleic acid of hepatitis C virus in step a)
encoding a complete NS3 coding region.

10 6. The protein produced according to claim 1,
said nucleic acid of hepatitis C virus in step a)
encoding a complete NS3 through NS5B coding region
comprising amino acid residues from 1027 to 3011 of
hepatitis C virus genome.

15 7. The protein produced according to claim 1,
wherein said expression system is a recombinant
baculovirus-insect cell expression system.

20 8. The protein produced according to claim 1,
wherein the extracted protein is purified by
immunoaffinity chromatography using antibodies specific
for hepatitis C virus proteins.

25 9. The protein produced according to claim 1,
having basal NTPase activity in the range of 0-200 min⁻¹
and RNA helicase activity greater than 0.001 min⁻¹.

30 10. The protein produced according to claim 1,
having basal NTPase activity less than 150 min⁻¹ and
RNA helicase activity greater than 0.005 min⁻¹.

35 11. A process for preparing native, authentic,
enzymatically active NTPase/RNA helicase protein
comprising the steps of:

 a) expressing an NTPase/RNA helicase
 encoding nucleic acid of hepatitis virus
 in a eukaryotic expression system such

that a complete, authentic and native
NTPase/RNA
helicase protein is synthesized, said
authentic and native NTPase/RNA helicase
protein comprising amino acids 1027-
1657;

- b) extracting NTPase/RNA helicase protein
from said eukaryotic expression system
in an enzymatically active form of said
protein; and
- c) purifying said NTPase/RNA helicase
protein such that the enzymatically
active form of said protein is
maintained.

12. The process according to claim 11, said nucleic
acid of hepatitis C virus in step a) corresponding to a
complete NS3 coding region.

13. The process according to claim 11, said nucleic
acid of hepatitis C virus in step a) corresponding to a
complete NS3 through NS5B coding region.

14. A native, authentic, enzymatically active
NTPase/RNA helicase protein product produced by a
process comprising the steps of:

- a) expressing a nucleic acid sequence in an
expression system, thereby producing an
enzymatically active, native, full
length hepatitis C virus NTPase/RNA
helicase protein that comprises the
amino acid residues having sequence
numbers from 1027 to and including 1657,
wherein said expression system is a
eukaryotic expression system;

- b) extracting said protein from said expression system, such that the extracted protein is in an enzymatically active form;
- c) purifying said extracted protein from step b) such that the purified protein is an enzymatically active, native, full-length hepatitis C virus NTPase/RNA helicase protein.

15. A method for assaying a compound for anti-viral activity against hepatitis C virus comprising:

- a) providing enzymatically active, native, authentic hepatitis C virus NTPase/helicase protein;
- b) contacting said protein with a compound suspected of inhibiting helicase activity; and
- c) measuring inhibition of the helicase activity in said protein by said compound.

16. A method for assessing a compound for anti-viral activity against a flavivirus, comprising:

- a) providing enzymatically active, native, authentic flavivirus helicase protein;
- b) contacting said protein with a compound suspected of inhibiting helicase activity; and
- c) measuring inhibition of the helicase activity in said protein by said compound.

17. A method as claimed in claim 15, wherein multiple compounds are assayed simultaneously.

18. A method for assaying a compound for anti-viral activity against hepatitis C virus comprising;

a) providing an enzymatically active, hepatitis C virus NTPase/RNA helicase protein;

b) providing a partially duplex substrate in which both strands are RNA and at least two nucleotides at the 3' end of at least one RNA strand are not involved in base pairing and at least one of said RNA strands is detectably labeled;

c) exposing said NTPase/RNA helicase protein to said partially duplex RNA substrate in the presence of a putative antiviral compound;

d) capturing any detectably labeled single stranded release strand product of the interaction between said RNA helicase protein and said substrate with a capture system comprising a specific binding pair, one member of said specific binding pair being conjugated with an oligonucleotide having a nucleotide sequence complementary to said detectably labeled release strand and the other member of said specific binding pair being affixed to a solid support; and

e) quantitating detectable label present in said release strand, as a measure of the anti-viral activity of said compound.

19. A method according to claim 18, wherein the other member of said specific binding pair is affixed to a mobile solid support.

20. A method according to claim 18 in which said oligonucleotide of said capture system is DNA.

21. A method according to claim 20 in which said capture system comprises said oligonucleotide conjugated with biotin and agarose beads coated with streptavidin or a derivative thereof.

22. A method as claimed in claim 18, wherein multiple compounds are assayed simultaneously.